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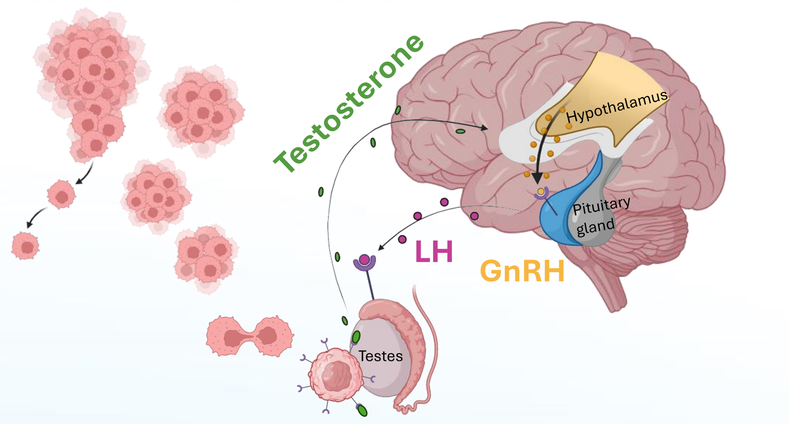
# Leuprorelin Peptide Drug Complexes with Permeability Enhancers to Increase Bioavailability

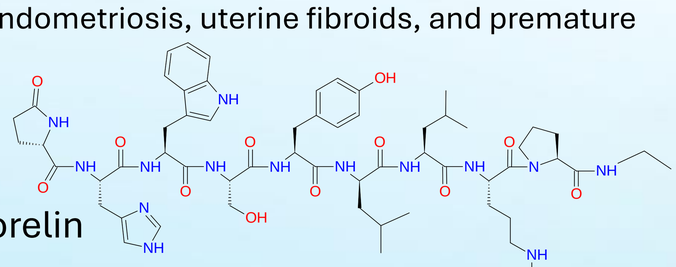
Adel Penhasi[✉,1,2], Zeina Zaatari[1], Moustafa Ghanyem[1], Deborah E. Shalev[✉,1,3]

*[1]Department of Pharmaceutical Engineering, Azrieli College of Engineering Jerusalem, Israel, [2]Department of Research and Development, Polycaps Holdings Ltd, Atidim Technology Park, Tel Aviv, Israel and [3]Wolfson Centre for Applied Structural Biology, The Hebrew University of Jerusalem, Israel*

One in eight men will develop prostate cancer during their lifetime and it is the second-leading cause of cancer death in men in the world.

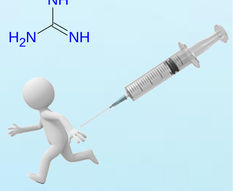


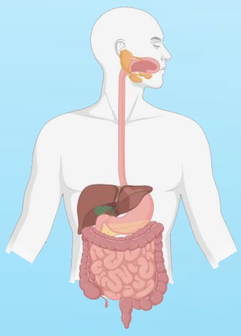

Testosterone-dependent prostate cancer is currently treated by desensitizing the gonadotropin hormone-releasing hormone (GnRH) receptor which stops testosterone production. This is done with the GnRH agonist, leuprorelin. The drug is also used to treat breast cancer, endometriosis, uterine fibroids, and premature puberty.



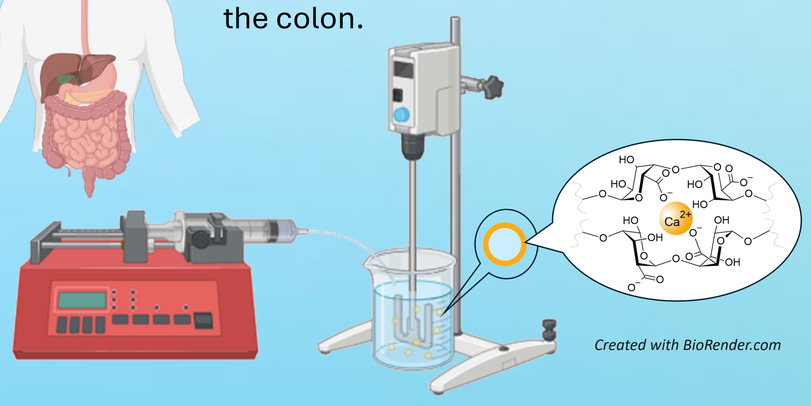
Leuprorelin

Leuprorelin, and all peptide drugs, suffer from extreme metabolic and chemical instability, since the body identifies them as food. Therefore, the drug is administered by injection, which reduces compliance.

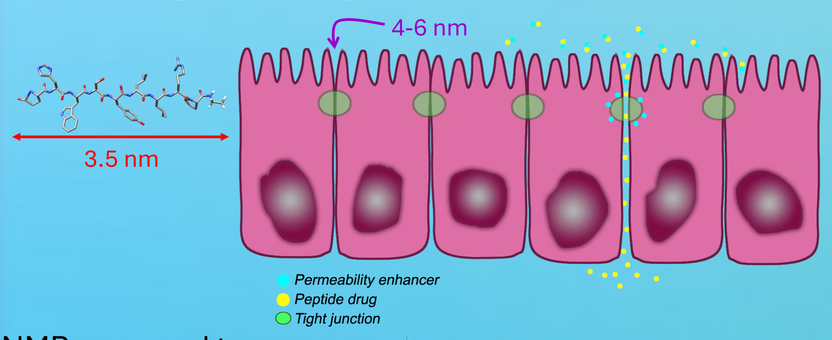




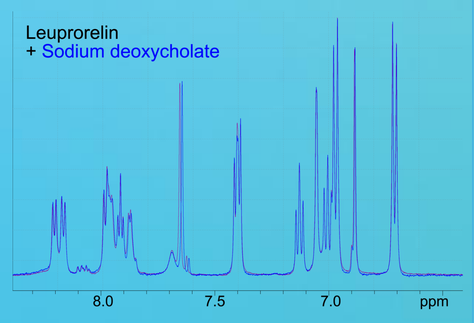
An oral formulation of leuprorelin is being prepared using alginate with calcium crosslinking to protect it until it reaches the colon.



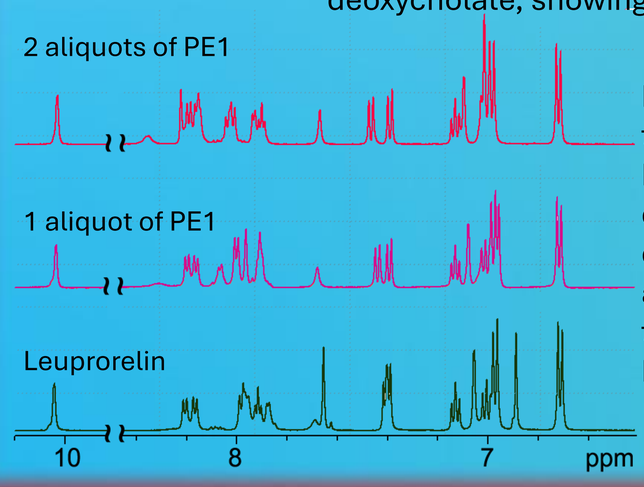

Permeability enhancers are necessary for leuprorelin to traverse the membrane of the gastrointestinal tract since it is too large to do so by itself.




NMR was used to measure interactions between leuprorelin and permeability enhancers: Six known permeability enhancers showed significant interactions, and five showed no significant interactions.

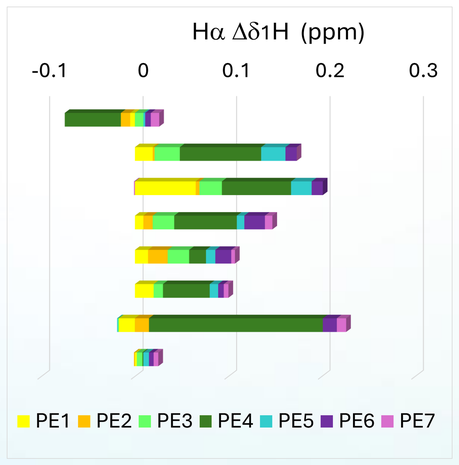


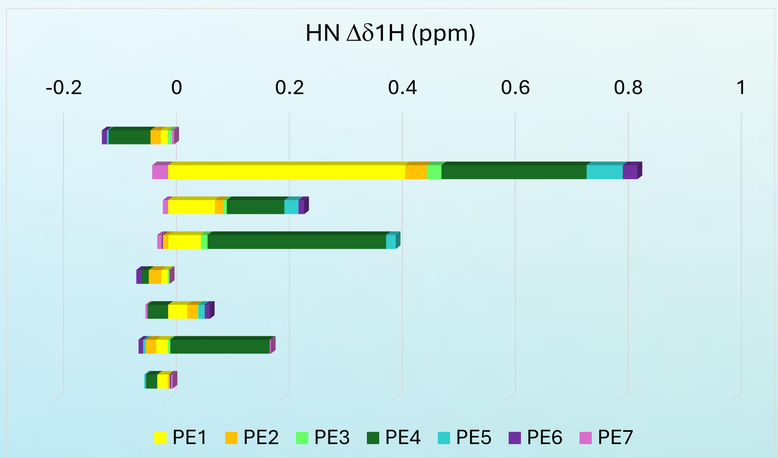

Leuprorelin titration with sodium deoxycholate, showing no interaction.

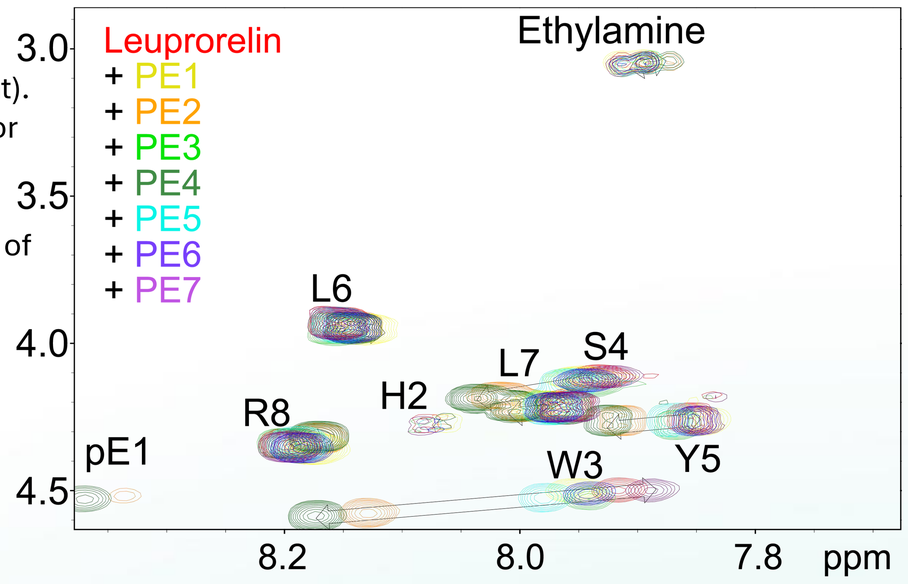


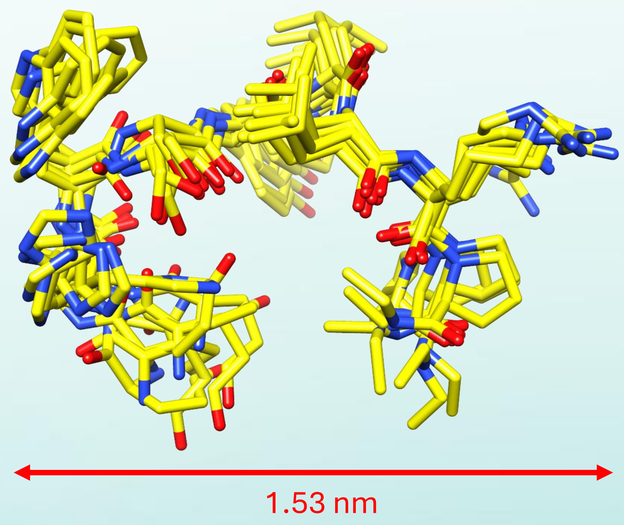

Leuprorelin titration with PE1 showing changes in chemical shift as a function of titration in $^1$H-NMR spectra.

Leuprorelin titration with all permeability enhancers (PE): 2D NMR $^1$H COSY spectra (right). As seen below, the changes in chemical shift for the various permeability enhancers were significant both for HN and Hα resonances, indicating a significant degree of complexation of leuprorelin with the permeability enhancer.

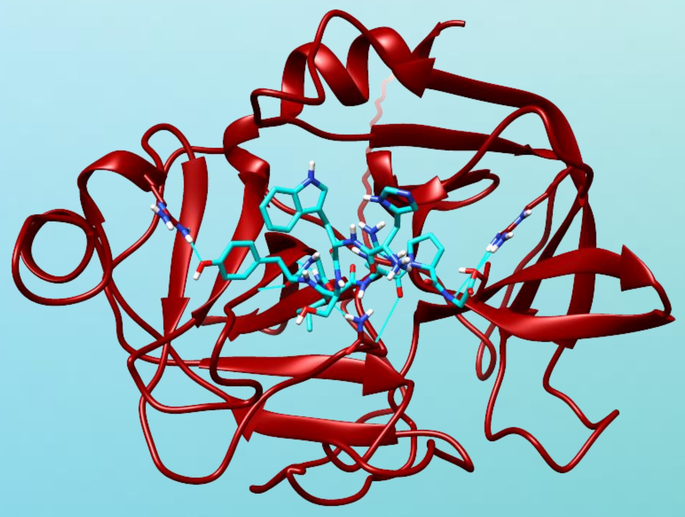


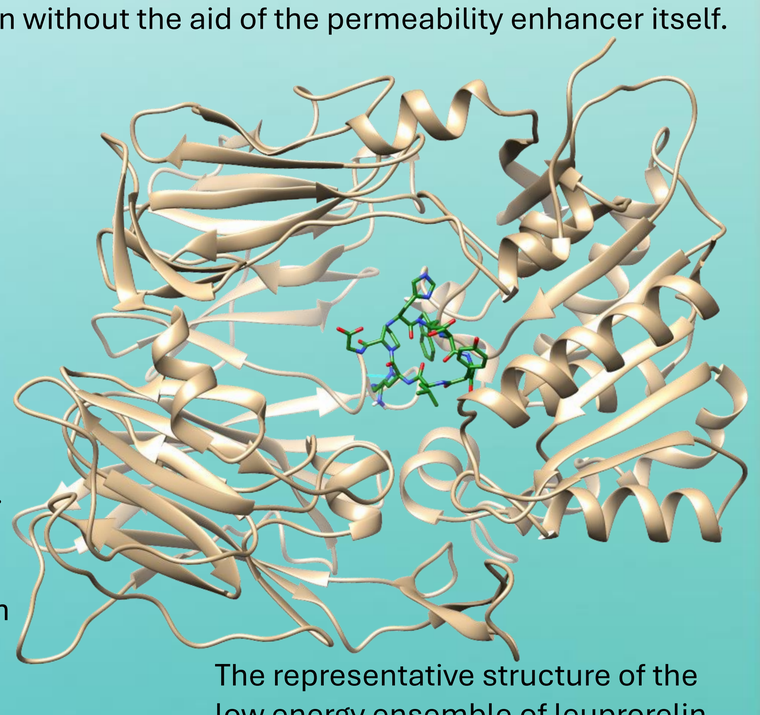



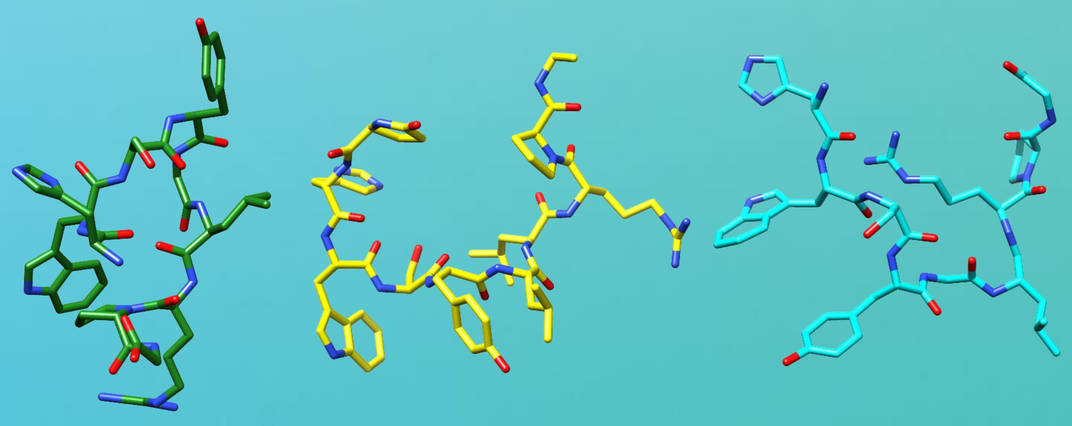




The low energy ensemble of leuprorelin (yellow) bound to PE1 (not shown) showing a structure that has a radius of 1.53 nm, which is significantly smaller than the 3.5 nm measured for the extended peptide and also smaller than the 4-6 nm passage through the tight junctions, even without the aid of the permeability enhancer itself.



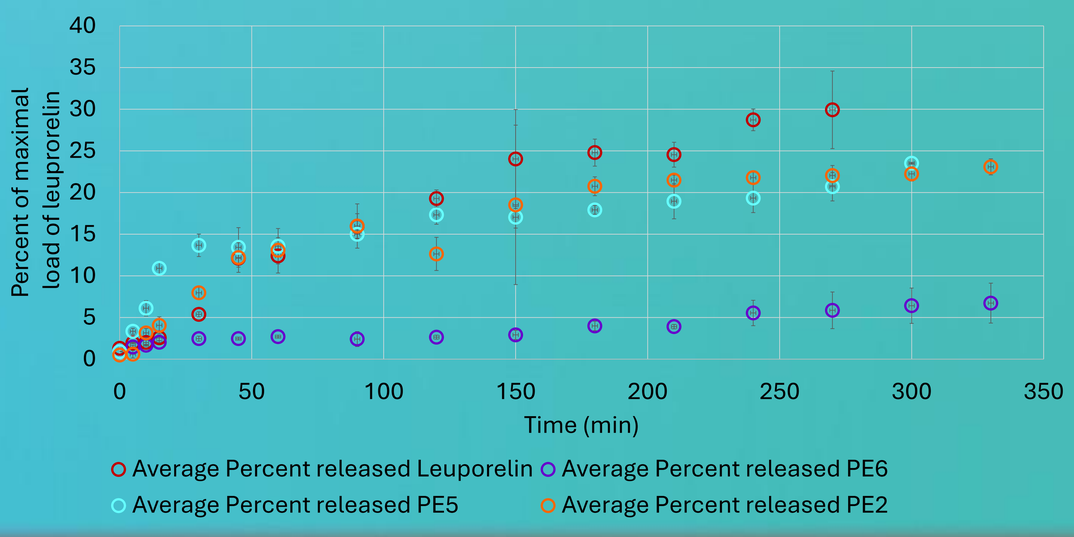
AlphaFold2-multimer on ColabFold (Mirdita, M., Schütze, K., Moriwaki, Y. et al. ColabFold: making protein folding accessible to all. *Nat Methods* **2022**) was used to calculate (above) the complex of GnRH hormone peptide (cyan) and Chymotrypsin C (red), and (right) the GnRH hormone peptide (green) in complex with dipeptidyl peptidase (tan).





The representative structure of the low energy ensemble of leuprorelin bound to PE1 (yellow) compared to the structure of GnRH hormone from the complex with chymotrypsin C (cyan), and GnRH from the complex with dipeptidyl peptidase (green), showed significant differences in structure that should prevent the PE-bound peptide from entering the enzyme binding sites.

Release profiles (below) from calcium-crosslinked alginate microsphere formulations of leuprorelin bound to permeability enhancers PE2, PE5 and PE6 showed significant extended release relative to leuprorelin itself.




**Conclusion**: NMR can be used to identify and qualify the interaction between leuprorelin and the various permeability enhancers. The upfield shift seen for the interaction with PE6 may suggest enhanced shielding that correlates the degree of binding and subsequent extended release profile.